High-throughput epitope identification for snakebite antivenom

Engmark, Mikael; De Masi, Federico; Laustsen, Andreas Hougaard; Gutiérrez, José María; Lomonte, Bruno; Andersen, Mikael Rørdam; Lund, Ole

Publication date:
2015

Document Version
Publisher's PDF, also known as Version of record

Citation (APA):

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.
High-throughput epitope identification for snakebite antivenom

Mikael Engmark¹, Federico De Masi¹, Andreas Hougaard Laustsen², José María Gutiérrez³, Bruno Lomonte³, Mikael Rørdam Andersen¹, and Ole Lund¹

(1) Department of Systems Biology, Technical University of Denmark
(2) Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen
(3) Instituto Clodomiro Picado, Facultad de Microbiología, Universidad de Costa Rica

Correspondence: mikael@bio.dtu.dk

Introduction

Insight into the epitopic recognition pattern for polyclonal antivenoms is a strong tool for accurate prediction of antivenom cross-reactivity and provides a basis for design of novel antivenoms. In this work, a high-throughput approach was applied to characterize linear epitopes in 966 individual toxins from pit vipers (Crotalidae) using the ICP Crotalidae antivenom. Due to an abundance of snake venom metalloproteinases and phospholipase A₂s in the venom used for production of the investigated antivenom, this study focuses on these toxin families.

Objectives

- Identify epitopes in toxins used in immunization
- Characterize tolerated amino acid substitutions in identified epitopes
- Predict cross-reactivity of antivenom

Immunization mixture

<table>
<thead>
<tr>
<th>Venom type</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bothrops</td>
<td>20%</td>
</tr>
<tr>
<td>Lachesis</td>
<td>20%</td>
</tr>
<tr>
<td>Crotalus</td>
<td>20%</td>
</tr>
<tr>
<td>Vipera</td>
<td>20%</td>
</tr>
<tr>
<td>Other</td>
<td>20%</td>
</tr>
</tbody>
</table>

Epitopes locate to surface regions

To identify epitopes the observed peptide specific signal intensities were mapped back to the amino acid sequence of each pit viper toxin. Using two or more overlapping 15-mer peptides with median signals above 20 AU, epitope core sequences were localized and subsequently mapped to crystal structures or homology models. As examples, P-I metalloproteinase and Lys49-phospholipase A₂ from Bothrops asper (venom used in antivenom production) are presented here.

Conclusions

- Custom-designed high density peptide microarray technology enables parallel automated identification of epitopes in hundreds of toxins.
- Integrating multiple sequence alignment allows investigation of the effect of epitope variation on antivenom recognition.
- Cross-reactivity of antivenom is correlated to the degree of conservation in toxin epitopes and flanking residues.

Acknowledgement

The peptide microarray experiments were performed at Schaefer N. Copenhagen. We would like to thank Claus Schauer, Christian Akeb Hansen, and Jens Kongskov for experimental setup and support. We further thank the Novo Nordisk foundation for financial support (grant number: NNF13OC001613).